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Discovery

Larvicidal activity of biologically synthesised silver nanoparticles against dengue vector *Aedes Aegypti* (Culicidae)

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ABSTRACT

Silver nanoaparticles have important applications in the field of biology. Stable silver nanoparticles were synthesized by biological reduction method. The objective of the present investigation was designed to determine the larvicidal activity of silver nanoparticles synthesized from aqueous leaf extract of *Cleistanthus collinus* against the larvae of *Aedes aegypti* (*Ae. aegypti*). *Ae. aegypti* larvae were exposed to varying concentrations of aqueous extract of *C. collinus* and synthesized silver nanoparticles for 24 h as per WHO protocols. Percentage of larval mortality was recorded. The synthesized nanoparticles exhibited significant larvicidal activity. This method is considered as an innovative alternative approach using green nanochemistry technique to control dengue vector parasites of *C. collinus* leaf mediated synthesized silver nanoparticles.

Keywords: Silver nanoparticles, larvicidal, *Aedes aegypti, Cleistanthus collinus*

1. INTRODUCTION

India is endemic to mosquito-borne diseases due to favorable ecological conditions. Insect- transmitted diseases are major health problems in tropical regions. *Aedes aegypti* (*Ae. aegypti*) occurs in Asia, Africa and Central and South America. It transmits virus of Flavivirus genus, etiologic agents of human diseases like dengue and yellow fever (Samidurai et al., 2009). Various synthetic products and devices have been designed to combat resistance developed by various mosquito species. Silver nanoparticles are emerging as one of the fastest growing materials due to their unique physical, chemical and biological properties; small size and high specific surface area. Biological synthesis of nanoparticles has received increased attention due to a growing need to develop environmentally benign technologies in material synthesis. Several plant species have been utilized in this regard.

Cleistanthus collinus (*C. collinus*) (Roxb.) is a toxic plant belonging to the family Euphorbiaceae and it grows in the dry forests of southern and central parts of India, Malaysia and Africa. It is commonly called as "garari" in Hindi, "oduvan" in Tamil, "vadise" in Telugu and "nilapala" in Malayalam. Many parts of the plant were reported as toxic and the aqueous extract of the crushed leaves of this plant are used as cattle and fish poison, abortifacient, suicidal and homicidal agents. The alcoholic extract of the leaves, roots and fruits of *C. collinus* are used to treat gastro intestinal disorders and it also possess anticancer activity. Further, the plant also possesses insecticidal properties against the red flour beetle, *Tribolium castaneum* and is used as insecticides in rice fields (Harwansh et al., 2010; Gupta et al., 2010). The leaf extracts of this plant exhibited insecticidal properties such as antifeedant and insect growth regulatory against the armyworm, *Spodoptera litura* (Selvamuthukumaran and Arivudainambi, 2008a & b; Selvamuthukumaran and Arivudainambi, 2010). Recently, Arivoli and Samuel (2012) reported that solvent crude extracts of *C. collinus* showed exhibited larvicidal activity against *Anopheles stephensi*. The use of environmentally benign materials such as silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for larvicidal application. Therefore the present study was carried out to determine the larvicidal activity of biologically synthesised silver nanoparticles from *C. colliuns* against dengue vector *Ae. aegypti*.

2. MATERIALS AND METHODS

2.1. Chemicals used

Silver nitrate (AgNO3) crystal extra pure was procured from Merck, Germany. Normal saline, double distilled water and de-mineralized water were used throughout the experiments.

2.2. Preparation of plant extracts

The leaves of *C. collinus* were cut into small pieces and the aqueous extract was prepared by mixing 10 g of dried leaves powder with 500 mL of water (boiled and cooled distilled water) with constant stirring on a magnetic stirrer (Minjas and Sarda 1986). The suspension of dried leaves powder in water was left for 3 h, filtered through Whatman no. 1 filter paper, and the filtrate was stored in amber colored air tight bottle at 10°C and used within a week.

2.3. Synthesis of Silver Nanoparticles

1mM $AgNO_3$ solution was prepared and stored in amber colour bottle. 5ml of leaf extracts was taken in conical flask separately and to this 50ml of 1mM $AgNO_3$ solution was added drop wise with constant stirring at 50-60 ºC and observed the colour change. The colour change of the solution was checked periodically then the conical flask was incubated at room temperature for 48 hours. The colour change of the leaf extract from yellow to dark brown indicated the silver nanoparticles synthesis from the plant extracts. All the nanoparticles preparation was made at Department of Biotechnology, PRIST University, Thanjavur, Tamilnadu, India.

2.4. Mosquito larvicidal bioassay

Standard methods for testing biologically synthesized nanoparticle toxicity and the susceptibility of mosquito larvae to insecticides was performed as stipulated by WHO (WHO, 1996). The larvicidal bioassay was performed at a room temperature of 27±10 ºC at Entomology Research laboratory, Department of Zoology, Arignar Anna Government Arts College, Musiri, Tamilnadu, India. Randomly, twenty (20) 4^{th} instar larvae were placed into 200 ml of sterilized double-distilled water and set in an environmental chamber at 27°C with a photoperiod 16:8-h light/dark cycle. The effectiveness of silver nanoparticles as mosquito larvicides was determined from all the ten 4th instar larvae with exposure to time periods. The larvae were separated into 4 small specimen bottles containing 25ml distilled water and the larvae were then exposed to each of the concentrations of the extracts in a final volume of 245ml distilled water taken in 500ml bowls. The nanoparticle solutions were diluted using double distilled water as a solvent according to the desired concentrations (5.0, 4.0, 2.0, 1.0, and 0.5 mg/L). At each tested concentration, four replicates and a control

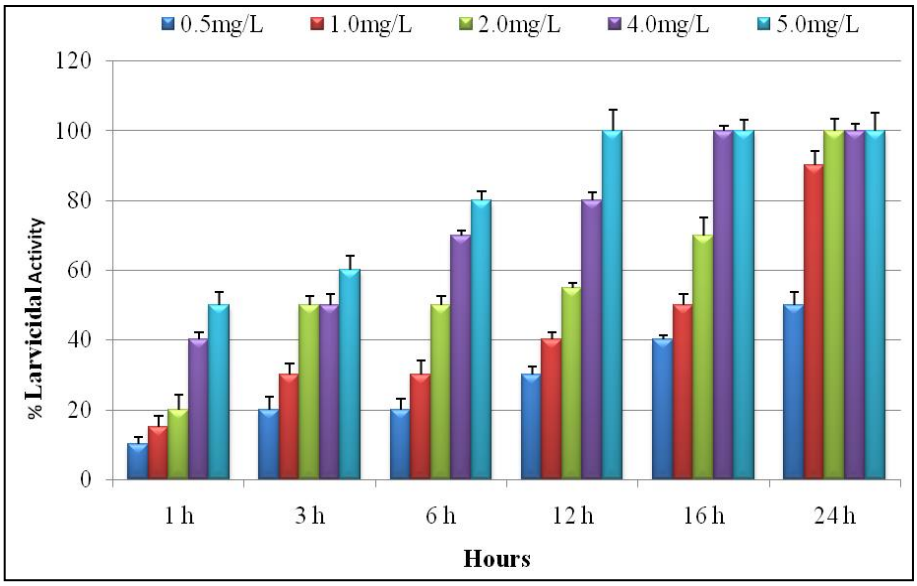

Figure 1

Survival percentage of *Aedes aegypti* larvae after exposure to different concentrations of silver nanoparticles

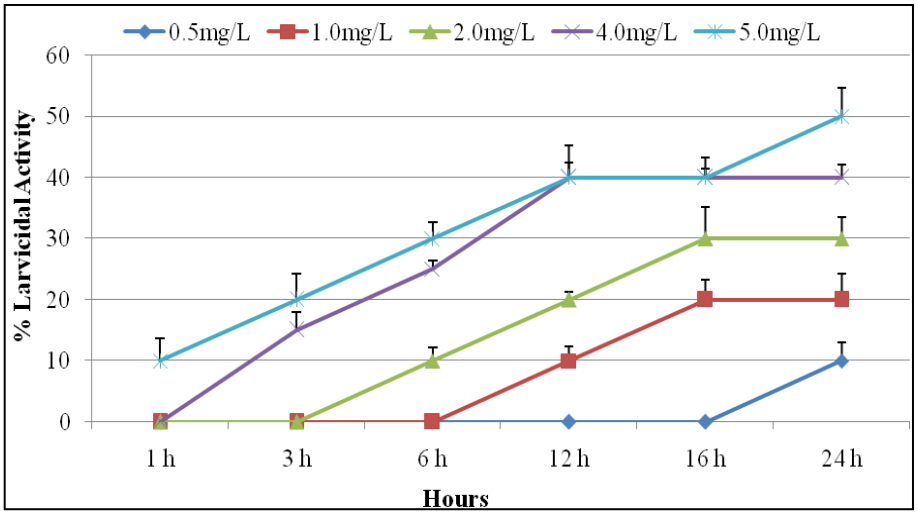

Figure 2

Survival percentage of *Aedes aegypti* larvae after exposure to different concentrations of aqueous extracts

(aqueous plant extracts) were tested for anti–larval effects. The larval mortalities were assessed to determine the acute toxicities on 4th instar larvae of *Ae. aegypti* at intervals of 1, 3, 6, 12, 16, and 24 hours of exposure. The number of dead larvae was counted from the 1st hour of exposure, and the percentage of mortality was reported from the average of four replicates. The larval mortality data were corrected for control mortality by the formula of Abbott (Abbott, 1925).

3. RESULTS AND DISCUSSION

The use of plant product chemistry coupled with nanotechnology that reducing mosquito populations at the larval stage can provide many associated benefits to vector control. Since silver nanoparticles are considered to be potential agents for various biological applications and also as a mosquito larvicidal agents. The larvicidal activity of biosynthesis of silver nanoparticles from *C. collinus* against larvae of *Ae. aegypti* is presented in figures 1 and 2. The data obtained from the present study clearly indicate that silver nanoparticles could provide significant larval mortality of *Ae. aegypti*. Greater mortality is seen in larvae treated with silver nanoparticles compared to aqueous (water) extract. Higher concentrations of aqueous plant extract showed 50% larval mortality observed at 5.0mg/l on 24 hours of exposure. The nanoparticle at 1.0 mg/l slightly decreased the survival of larvae to 50% after 16 hours of exposure, while 100% mortality of the larval population was observed in a concentration of 5.0mg/l nanoparticles within 12 hour. The nanoparticle of 1.0mg/l killed the larvae slowly and nearly 90% mortality was found after 24 hours of exposure. The maximum efficacy was observed in 5.0 mg/l of silver nanoparticles. The mechanism which causes the death of the larvae could be the ability of the nanoparticles to penetrate through the larval membrane. The silver nanoparticles in the intracellular space can bind to sulphur-containing proteins or to phosphorus containing compounds like DNA, leading to the denaturation of some organelles and enzymes (Rai et al., 2009). Sarah et al., (2012) reported that silver nanoparticles synthesized from aqueous leaf extract of *Hibiscus rosasinensis* against the larvae of *Ae. albopictus* mosquito showed high larval mortality compared to aqueous extract and subsequently, the decrease in membrane permeability and disturbance in proton motive force causes loss of cellular function and finally cell death.

4. CONCLUSION

The prospect of utilizing plant based products for synthesizing silver nanoparticles and testing its efficacy in controlling mosquitoes as larvicides is a recent phenomenon facilitating the development of a more potent and eco-friendly pesticide. Identification of the bioactive principles involved and their mode of action and field trials are necessary to recommend an effective formulation as an anti-mosquito control programs.

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REFERENCE

1. Abbott WS. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomol., 1925,* 18, 265-267
2. Arivoli S, Samuel T. Larvicidal efficacy of *Cleistanthus collinus* (Roxb.) (Euphorbiaceae) leaf extracts against vector mosquitoes (Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine*. 2011, S281-S283
3. Gupta R, Vairale MG, Deshmukh RR, Chaudhary PR, Wate SR. Ethnomedicinal uses of some plants used by Gond tribe of Bhandara district, Maharashtra. Indian J Tradit Knowledge, 2010, 9(4), 713-717
4. Harwansh RK, Dangi JS, Jha AK, Deshmukh R. Effect of medicinal plant Garari (Cleistanthus collinus) Family: Euphorbiaceae against red flour beetles (*Tribolium castaneum*). *J Pharm Res,* 2010, 3(5), 965-968
5. Minjas JN, Sarda RK. Laboratory observations on the toxicity of Swartzia madagascariens (Leguminaceae) extract to mosquito larvae. *Trans R Soc Trop Med Hyg*, 1986, 80, 460–461
6. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials, *Biotechnol.Adv.,*2009, 27, 76-83
7. Samidurai K, Jebanesan A, Saravanakumar A, Govindarajan M, Pushpanathan T. Larvicidal, ovicidal and repellent activities of *Pemphis acidula* Forst. (Lythraceae) against filarial and dengue vector mosquitoes. *Acad J Entomol*. 2009, 2(2), 62-66
8. Sareen Sarah J, Pillai Raji K, Chandramohanakumar N, Balagopalan M. Larvicidal Potential of Biologically Synthesized Silver Nanoparticles against *Aedes Albopictus*. *Research Journal of Recent Sciences*. 2012, 1 -52-56
9. Selvamuthukumaran T, Arivudainambi S. Insect growth regulatory action of certain leaf fractions of *Cleistanthus collinus* (Roxb.) Benth (Family: Euphorbiaceae) against *Spodoptera litura* Fab. (Noctuidae: Lepidoptera). Hexapoda. 2008a, 15(2), 125-127
10. Selvamuthukumaran T, Arivudainambi S. Insecticidal properties of *Cleistanthus collinus* (Roxb.) Benth (Family: Euphorbiaceae) against *Spodoptera litura* Fab. (Noctuidae: Lepidoptera). *Plant Arch*. 2008b, 8(2), 683-685
11. Selvamuthukumaran T, Arivudainambi S. Dose dependant differential anti insect activity of lactone glycoside a potent plant derived molecule. *J. Biopest*. 2010, 3(1), 259-264
12. WHO. Report of the WHO informal consultation on the evaluation on the testing of insecticides CTD/WHO PES/IC/., 1996, 96(1), 69